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Forest  
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## FAX TRANSMITTAL COVER SHEET

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SUBJECT: Genetics

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DATE SENT: 4/16/08

NUMBER OF PAGES TO FOLLOW: 4





# The University of Montana

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January 15, 2001

Lee Brundin  
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Lee:

We have completed genetic analysis of the following samples:

Table 1. Summary of results.

Location	Received	N <sup>a</sup>	# markers <sup>b</sup>	Species ID <sup>c</sup>	Power (%) <sup>d</sup>	% WSCT <sup>e</sup>
Elliot Creek	9/10/99	15	-	WSCT	84	100
Rainy Creek, on Owens	9/10/99	10	-	WSCT	70	100
Rainy Creek, on Plum Creek	9/10/99	14	-	WSCT	82	100
Blue Creek, upper	9/10/99	15	4	WSCT x YSCT	70	95
Noisy Creek	9/10/99	16	6	WSCT x RBT	85	93
Shafer Creek, upper	9/10/99	15	6	WSCT x RBT	84	92
Swamp Creek, at Poker Hill	9/10/99	18	6	WSCT x RBT	89	86
Shafer Creek, lower	9/10/99	13	6	WSCT x RBT	79	83
Smearl Creek	9/10/99	15	6	WSCT x RBT	84	80
Trail Creek	9/10/99	15	6	WSCT x RBT	84	77
Crazyman Creek	9/10/99	16	6	WSCT x RBT	85	69
Carrie Creek	9/10/99	7	6	WSCT x RBT	57	69
Alexander Creek	9/10/99	15	6	WSCT x RBT	83	17
Doak Creek	9/10/99	14	6	WSCT x RBT	82	9

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<sup>a</sup>Number of samples successfully analyzed.

<sup>b</sup>Number of markers analyzed that are diagnostic for the non-native species (second species listed under Species ID).

<sup>c</sup>Single species code is used when the entire sample possessed alleles from that species only. However, it must be noted that we cannot definitively rule out the possibility that some or all of the individuals are hybrids. We merely have not detected any non-native alleles at the loci examined (see Power %). Codes: WSCT = westslope cutthroat trout (*Oncorhynchus clarki lewisi*); RBT = rainbow trout (*O. mykiss*); YSCT = Yellowstone cutthroat trout (*O. clarki bouvieri*). Species codes separated by "x" indicate apparent hybridization between those species.

<sup>d</sup>Number corresponds to the percent chance we have to detect 1% hybridization given the sample size and number of diagnostic markers used (a sample size of 25 is required to yield a 95% chance to detect 1% hybridization of rainbow or Yellowstone cutthroat trout into a westslope trout population).

<sup>e</sup>Indicates the genetic contribution of westslope cutthroat trout to the sample assuming random mating proportions.

Note: For further details on each sample, see the "Sample Details" section below.

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### Brief Description of Methods:

Polymerase chain reaction (PCR) amplification of paired interspersed nuclear DNA elements (PINES) was used to determine each fish's genetic characteristics at multiple regions of the nuclear DNA. This method produces DNA fragments that can be used to distinguish between various cutthroat trout subspecies (*Oncorhynchus clarki spp.*), rainbow trout (*O. mykiss*) and their hybrids. The presence of a PINE marker is dominant to absence. First-generation ( $F_1$ ) hybrids will have all the diagnostic markers characteristic of the two hybridizing species. Backcrossed ( $F_2+$ ) individuals will possess some, but not all, markers characteristic of both parental species. The appearance of a marker indicates the individual is either heterozygous or homozygous for that marker, which precludes us from directly calculating allele frequencies. However, in order to provide comparative values, we have assumed the samples conform to random mating expectations in order to estimate the average genetic contribution from each species.

It is critical to note that in all hybrid swarms, regardless of the percent contribution from the non-native species, all individuals are of hybrid origin, even those that appear "pure" at our diagnostic loci. It is not possible to "rescue" pure individuals from these populations as they likely do not exist. Due to the random reshuffling of alleles during sexual reproduction, many individuals will appear pure for one or the other parental species due to the limited number of marker loci used. It has been shown that 4-6 markers are adequate to provide coarse classification of hybridization, but upwards of 70 markers are required to discriminate between pure individuals (if they exist) and backcrossed individuals in hybrid swarms (Boecklen and Howard 1997).

### Literature Cited:

Boecklen WJ, and Howard DJ (1997) Genetic analysis of hybrid zones: numbers of markers and power of resolution. *Ecology* 78 (8) pp. 2611-2616.

### Sample Details:

**Elliot Creek:** Markers characteristic of westslope cutthroat trout only were detected in the 15 samples. It should be noted, however, that with a sample size of 15, we only have an 84% chance to detect 1% hybridization.

**Rainy Creek, on Owens:** Markers characteristic of westslope cutthroat trout only were detected in the 10 samples. It should be noted, however, that with a sample size of 10, we only have a 70% chance to detect 1% hybridization.

**Rainy Creek, on Plum Creek:** Markers characteristic of westslope cutthroat trout only were detected in the 14 samples. It should be noted, however, that with a sample size of 14, we only have an 82% chance to detect 1% hybridization.

**Blue Creek:** These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid-origin, even those that appear "pure" at our diagnostic loci. All markers characteristic of westslope cutthroat trout were detected in the 15 samples. However, four of these individuals also possessed one marker, and one possessed two markers, characteristic of Yellowstone cutthroat trout. This



either indicates a small amount of Yellowstone hybridization into a westslope population, or the markers could simply be rare westslope cutthroat genetic variation that is electrophoretically indistinguishable from those characteristic of Yellowstone cutthroat trout. However, the latter possibility appears unlikely, as the Yellowstone markers were present at two independent loci. Assuming random mating proportions, the genetic contributions of Yellowstone cutthroat and westslope cutthroat in this sample are 5 and 95%, respectively.

*Noisy Creek:* These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers characteristic of both westslope cutthroat trout and rainbow were detected in these 16 samples. Nine individuals possessed only markers characteristic of westslope cutthroat. The remaining seven individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. Six of these seven hybridized individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. The remaining individual appeared to be an  $F_1$  hybrid. Assuming random mating proportions, the genetic contributions of rainbow trout and westslope cutthroat in this sample are 7 and 93%, respectively.

*Shafer Creek, upper:* These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers characteristic of both westslope cutthroat and rainbow trout were detected in these 15 samples. Eight individuals possessed all markers characteristic of westslope cutthroat only. The remaining seven individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. All seven of these hybridized individuals were at least second-generation hybrids as they did not possess all the alleles characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. Assuming random mating proportions, the contributions of rainbow trout and westslope cutthroat in this sample are 8 and 92%, respectively.

*Swamp Creek, at Poker Hill:* These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers characteristic of both westslope cutthroat trout and rainbow trout were detected in these 18 samples. Six individuals possessed all markers characteristic of westslope cutthroat. The remaining 12 individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. All of these hybridized individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. Assuming random mating proportions, the genetic contributions of rainbow trout and westslope cutthroat in this sample are 14 and 86%, respectively.

*Shafer Creek, lower:* These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers characteristic of both westslope cutthroat trout and rainbow trout were detected in these 13 samples. Five individuals possessed all markers characteristic of westslope cutthroat only, and one individual possessed all markers characteristic of rainbow trout only. The remaining seven individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. All of these hybridized individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. Assuming random mating proportions, the





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genetic contributions of rainbow trout and westslope cutthroat in this sample are 17 and 83%, respectively.

**Smearl Creek:** These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers characteristic of both westslope cutthroat trout and rainbow were detected in these 15 samples. Six individuals possessed all markers characteristic of westslope cutthroat. The remaining nine individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. All of these hybridized individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. Assuming random mating proportions, the genetic contributions of rainbow trout and westslope cutthroat in this sample are 20 and 80%, respectively.

**Trail Creek:** These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers characteristic of both westslope cutthroat trout and rainbow were detected in these 15 samples. Six individuals possessed all markers characteristic of westslope cutthroat. The remaining nine individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. Eight of these nine hybridized individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. The remaining individual appeared to be an  $F_1$  individual as it possessed all the markers characteristic of both species. Assuming random mating proportions, the genetic contributions of rainbow trout and westslope cutthroat in this sample are 23 and 77%, respectively.

**Crazyman Creek:** These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers characteristic of both westslope cutthroat trout and rainbow trout were detected in these 16 samples. Seven individuals possessed all markers characteristic of westslope cutthroat. The remaining nine individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. All of these hybridized individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. Assuming random mating proportions, the genetic contributions of rainbow trout and westslope cutthroat in this sample are 31 and 69%, respectively.

**Carrie Creek:** These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers characteristic of both westslope cutthroat trout and rainbow trout were detected in these seven samples. All individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. Six of these hybridized individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. The remaining individual appeared to be an  $F_1$  hybrid. Assuming random mating proportions, the genetic contributions of rainbow trout and westslope cutthroat in this sample are 31 and 69%, respectively.

**Alexander Creek:** These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. Markers

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characteristic of both westslope cutthroat trout and rainbow trout were detected in these 15 samples. Two individuals possessed markers characteristic of rainbow trout only. The remaining 13 individuals possessed markers in various quantities characteristic of both westslope cutthroat and rainbow trout. All of these hybridized individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. Assuming random mating proportions, the genetic contributions of rainbow trout and westslope cutthroat in this sample are 83 and 17%, respectively.

*Doak Creek:* These samples appear to have come from a hybrid swarm. All individuals in a hybrid swarm are likely of hybrid origin, even those that appear "pure" at our diagnostic loci. All markers characteristic of rainbow trout were detected in the 14 samples. Eleven individuals also possessed one or two markers characteristic of westslope cutthroat trout. All of these individuals were at least second-generation hybrids as they did not possess all the markers characteristic of both species, as is expected in first generation ( $F_1$ ) hybrids. Assuming random mating proportions, the genetic contributions of rainbow trout and westslope cutthroat in this sample are 91 and 9%, respectively.

Sincerely,

Marirose Spade

John K. Wenburg

Cc: Steve Carson

